



Comparison of PCR with stool culture for diagnosis of Asymptomatic Typhoid Carriers in stool samples collected in a tertiary care hospital in East India

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Abstract

Background: Typhoid is a disease affecting a large population throughout the world. Besides poverty, low education level and poor hygiene, contact with typhoid patient or a carrier has been identified as major risk factors. Approximately 2–5% of typhoid patients do not clear the infection and progress to carrier state. Stool culture is an important method of diagnosis in such cases. However the yield can be very low and many cases can be missed. Confirmed diagnosis and prompt treatment are essential to control the spread of typhoid.

Material and Methods: The study was conducted in the Department of Microbiology University Hospital, Institute of Medical Sciences, Banaras Hindu University, Varanasi, over a period of one year. Fifty stool samples from suspected asymptomatic carriers with history of clinically and serologically confirmed Typhoid one or more years ago were included in the present study. Stool Culture and PCR was done from the stool samples to confirm presence of *S. Typhi* carriage and results (yield) were compared.

Results and Discussion: Out of the 50 suspected Typhoid cases, 29 were positive for *S. Typhi* by PCR (58%) for flagellin gene of *S. Typhi*, while only 3 (6%) were positive by stool culture. PCR was found to be significantly better than stool culture ($p < 0.05$)

Conclusion: Stool culture remains a low yielding method which is time consuming and not very fruitful for detection of carriers. PCR for detection of *S. Typhi* from stool is a good confirmatory tool, especially for tertiary care setups where molecular diagnostic laboratory facilities are readily available.

Keywords: Enteric Fever, Typhoid carrier, *S. Typhi*, Nested PCR, Flagellin gene.

Introduction

Typhoid fever is a systemic infection caused by *Salmonella enterica* subspecies *enterica* serotype Typhi (*S. Typhi*), a Gram negative bacterium. A very similar but often less severe disease is caused by *S. Paratyphi* A, B, and sometimes C. *S. Typhi* a highly adapted humanspecific pathogen that

evolved about 50,000 years ago, has remarkable mechanisms for persistence in its host.^[1] This disease is now uncommon in the developed world where most of the case that occur are either acquired abroad or imported by immigrants. In U.K. typhoid fever has been brought very close to eradication with approximately one case per

1,00,000 population, which is perhaps the lowest incidence of typhoid fever in the world^[2].

Enteric fever (Typhoid fever) occurs throughout the world, and is a dreadful disease especially in developing countries where water supply and sanitation are substandard; it is transmitted through the fecal oral route by consumption of contaminated water and food. The presence of a convalescent patient or a carrier actively shedding the pathogen poses an increased risk for infection. Disease outbreak may occur from a unique source of food or carrier in the non endemic area^[3].

In disease endemic areas recent contact with a patient or carrier has been identified as a major risk factor^{[4][5]}, but other risk factors include poverty, low education level, poor hygienic conditions and water supplies, and eating outdoors at food stalls.

Following the resolution of disease, approximately 2–5% of typhoid patients fail to fully clear the infection within one year of recovery, instead progressing to a state of carriage^[6]. The basic requirements for establishment of long-term extra intestinal infection are likely to involve successful breach of the intestinal epithelial barrier, evasion of early innate immune-mediated killing, and localization to a permissive niche. The permissive niche in humans is primarily the biliary tract and gallbladder^[7].

Detection of carriers poses a difficult challenge and currently available diagnostic assays are limited. Standard practice has been to detect typhoid carriage through analysis of serial stool and urine samples, which is logistically difficult to perform at a population level and is associated with low sensitivity^[8].

The development of molecular methods for diagnosis of infectious diseases, has improved the sensitivity and specificity of diagnosis. One of the molecular methods, Polymerase chain reaction (PCR) is the most sensitive and rapid method to detect microbial pathogens in clinical specimens. Identification of the *S. Typhi* specific gene (*flagellin* gene) by PCR is said to be the better

technique and more sensitive^[9]. In particular, when the specific pathogen is difficult to culture in vitro or requires a long cultivation period, the diagnostic value of PCR appears very significant^[10].

This study was done to compare the results of Culture with that of PCR for detection of *S. Typhi* from Stool samples in suspected carriers, so as to help in formulating diagnostic algorithm in tertiary care hospitals where PCR is readily available.

Material and Methods

The study was conducted in the Department of Microbiology, Sir Sunderlal Hospital, Institute of Medical Sciences, Banaras Hindu University, Varanasi, over a period of one year. Fifty stool samples from suspected carriers with history of clinically and serologically confirmed Typhoid one or more years ago were included in the present study

Stool Culture to confirm presence of *S. Typhi*

Faecal culture is particularly valuable in carriers or patients on chloramphenicol therapy^[11]. About 5 g of freshly passed unpreserved stool was put in enrichment broth i.e. selenite F broth. Stool culture was subsequently done. Culture of faeces is a standard diagnostic technique and repeated specimens over 2-3 days, especially for detection of carriers, yield good results^[12].

Stool is usually plated on desoxycholate- citrate agar and also inoculated into fluid enrichment media such as tetrathionate or selenite broth. The limitation of liquid of medium is that the growth of fluid enrichment medium is subcultured appropriate medium for proper identification. Suspicious colonies from culture plates are tested directly for the presence of *Salmonella* O antigens by slide agglutination and subcultured to peptone water for determination of H antigen structure and for further biochemical analysis^[13].

PCR for *S. Typhi*: The stool specimens (3 to 5 g) from each of the study subjects were subjected to genomic DNA extraction using the method

described by Van Zwet et al. with slight modification to minimize PCR inhibitors. The additional steps included 3 to 5 g of stool added to 10 ml of 10% formal saline (40% [wt/vol] formaldehyde and 0.85% [wt/vol] NaCl) and mixed well to make a suspension. Following this, 3 ml of ether was added and centrifuged at 3,000 rpm for 5 min. to remove debris and supernatant was again centrifuged at 10000 rpm 10 min at 4°C. Pellet was subjected to DNA isolation^[14].

Primers which target the flagellin (*HI-d*) gene of *Salmonella* Typhi were obtained from SBS Gentech Co., Ltd China (Song *et al.*, 1993). And modified by Frankel^{[15][16]}.

Oligonucleotides were used for first round PCR to amplify a 458 base pair (bp) fragment.

ST1 (5'-ACT GCT AAA ACC ACT ACT-3') and ST2 (5'-TTA ACG CAG TAA AGA GAG-3'),

Oligonucleotides were used for second round PCR (Nested PCR) to amplify a 343 bp fragment ST3 (5'-AGA TGG TAC TGG CGT TGC TC-3') and ST4 (5'-TGG AGA CTT CGG TCG CGT AG-3')

Master Mix for 1st round PCR

25µl master mix for the first cycle of PCR was prepared and subjected to 35 cycles of PCR with following temperature and duration, The reaction procedure consisted initial denaturation at 94 °C for 5 min. followed by 35 cycles of denaturation 94 °C for 1 min., annealing at 57°C for 1 min, extension 72°C for 1 min, Final Extension 72°C for 7 min

Master mix for nested PCR

25 µl master mix for nested PCR contains primers i.e. ST3 and ST4 in place of ST 1 & ST2 and 1 µl product of 1st cycle was used as template. Thermal cycling was carried out as described for first-round PCR, except that the annealing temperature was set to 63°C

Detection of PCR product

10 µl of amplified product of nested PCR was electrophoresed on 1.5 % agarose gel electrophoresis at 100 volts for 5 min and then at constant 80 volts for 60 min with Tris Borate

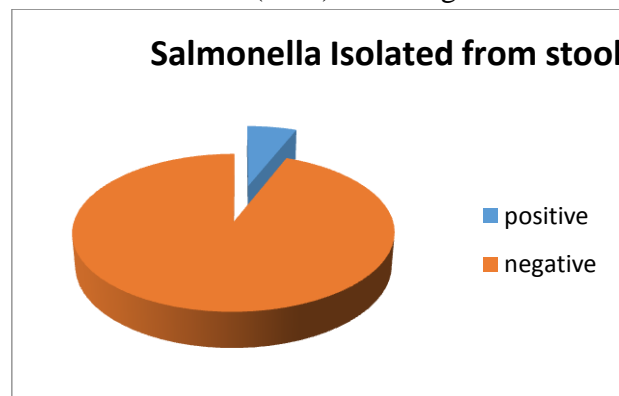
EDTA buffer (HiMedia, RM 273 Mumbai, India), Molecular marker (100 bp DNA Ladder, MBI Fermentas, Germany) were run concurrently. The gel stained with ethidium bromide were visualized under ultraviolet illumination and saving of image for the presence of 343 bp bands by using Multi Image Light Cabinet (Alpha Innotech Corporation, US).

Results

Isolation of *Salmonella* Typhi from stool specimen

| | No. | % |
|-----------------|-----|-----|
| Positive | 3 | 6% |
| Negative | 47 | 94% |

Table showed that 3 (6%) patients were positive for isolation and 47 (94%) were negative.

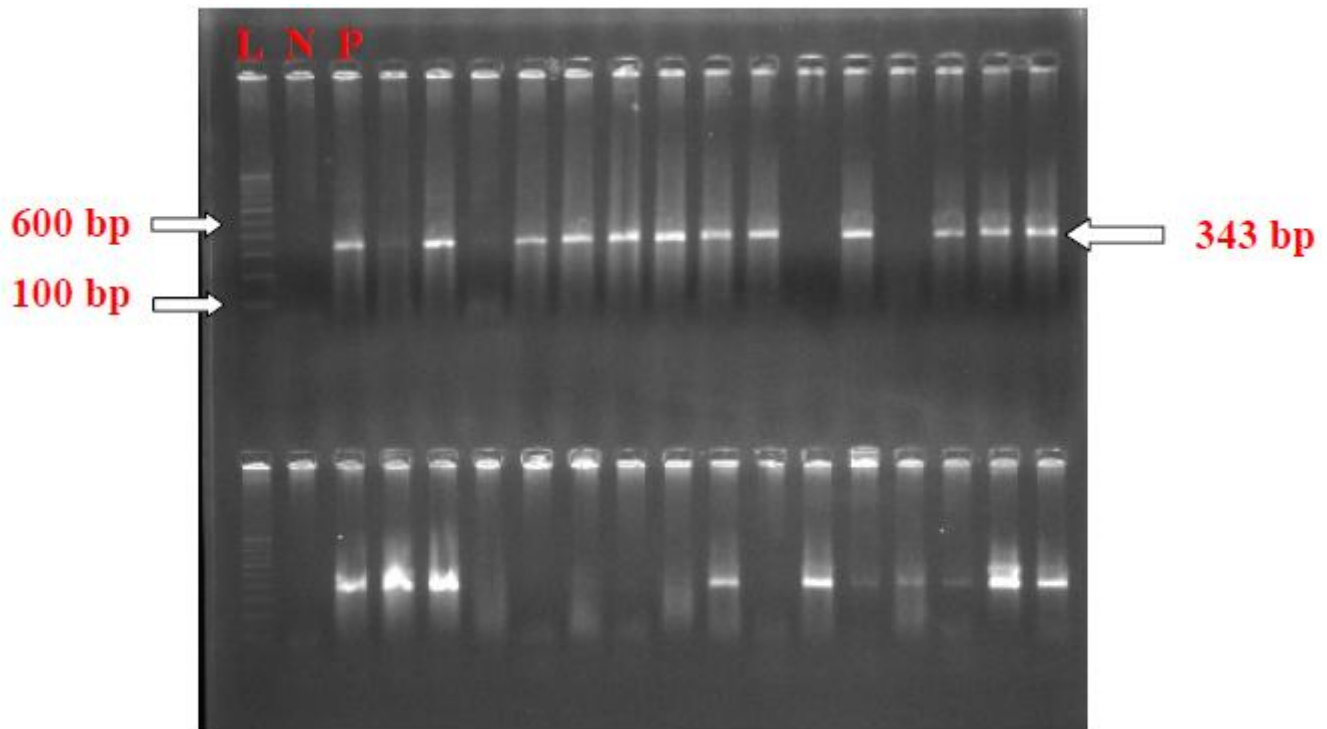


Detection of *Salmonella* Typhi in stool by PCR

| | No. | % |
|-----------------|-----|------|
| Positive | 29 | 58% |
| Negative | 21 | 42 % |

Of the total 50 suspected cases of typhoid fever, 29 (29/50; 58%) stool samples yielded positive amplicon of 343 bp after nested PCR specific for flagellin gene of *S.Typhi*.

PCR products were run on gel electrophoresis and image taken in multi image light cabinet.



nPCR for *Salmonella* TyphifliC gene (flagellin)
 L : Ladder (Molecular marker)
 N : Negtive control
 P : positive control

Discussion

Asymptomatic, chronic carriers of *Salmonella* Typhi have been reported from all over the world since a very long time. The most famous of carrier case reports date about 100 years back. Typhoid Mary, who was the subject of one of the first true epidemiological investigations of an infectious disease, was a chronic carrier of *S. Typhi* who remained asymptomatic spread the disease to many.^[17]

What has been lacking are studies to address how this bacterium uniquely establishes a persistent infection in this organ and how we can better identify, diagnose, and treat these carriers. These issues need to be solved, as carriers represent a reservoir for the continued spread of infection of *S. Typhi*. Recent molecular and patient-based studies have advanced the field, and provide a foundational basis for a greater understanding of this chronic human illness.^[18]

Parry et al report that detection rates from stool culture remains poor. Detection of carriers remains a big challenge. As much as one fourth of the carriers do not give any history of typhoid^[19]

A study done by Bokkenheuser V states that shedding of the organisms is intermittent and in low levels, methods for diagnosis of *S. Typhi* in feces of carriers by stool culture have many limitations. Stool culture is also not a good tool for large-scale screening as it may be difficult to perform at a population level and is associated with low sensitivity^[20].

This study found that out of the fifty suspected Typhoid cases, 29 were positive for *S. Typhi* by PCR (58%) for flagellin gene of *S. Typhi*, while only 3 (6%) were positive by stool culture. PCR was found to be significantly better than stool culture ($p < 0.05$)

Recovery of *S. Typhi* in bile, gallbladder stones, or tissue of afebrile individuals undergoing elective cholecystectomy is considered a gold standard of diagnosis, but this approach also has minimal public health utility because of its invasive nature. Molecular methods have also been employed to increase sensitivity, including fluorescent antibody techniques on fecal smears, but sensitivity has not surpassed culture^[21]. Polymerase chain reaction (PCR) amplification

for *S. Typhi* specific genes remains a good and cost effective diagnostic test if done on large scale. However stool sample has PCR inhibitors also which can hamper the sensitivity. Thus, this study suggests that further research must be done to improve the sensitivity by using PCR.

Conclusion

Stool culture remains a low yielding method which is time consuming and not very fruitful for detection of carriers. PCR for detection of *S. Typhi* from stool is a good confirmatory tool, specially for tertiary care setups where molecular diagnostic laboratory facilities are readily available.

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